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일회용 타액 배출기 재사용에 대한 미생물학적 연구

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Microbiological Investigation of Reusing Disposable Saliva Ejectors

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Abstract

Objective: Dental instruments are often designed for single use; however, some dental clinics resort to reusing instruments, including saliva ejectors. In this study, we aimed to investigate and compare various disinfection and sterilization methods for contaminated disposable salivary ejectors. **Methods:** Saliva ejectors contaminated with *Streptococcus mutans* were divided into five groups: negative control, positive control, cleaning, disinfection, and sterilization. Surface contamination and microbial culture tests were conducted using the adenosine triphosphate (ATP) test and colony-forming units (CFUs) counting, respectively. One-way ANOVA was used to assess the differences among groups. **Results:** There were no significant differences among the cleaning, disinfection, and sterilization groups based on immediate ATP test. However, CFU counts were significantly higher in the cleaning and disinfection groups compared to negative control group, while no microbial growth was observed in the sterilization group. **Conclusions:** This study demonstrated that saliva ejectors harbor bacteria capable of growth, even after cleaning with a toothbrush or disinfectant. Autoclaving proved to be the most effective sterilization method, eliminating microbial growth, and allowing for microbiological reuse. Future studies involving diverse microorganisms and different types of contaminants are recommended.

Key words: Dental instrument, Disinfection, Infection control, Saliva ejector, Sterilization

색 인: 소독, 치과 기구, 감염 관리, 타액 배출기, 멸균

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1. Introduction

Many instruments in dental settings are designed for single use due to exposure to blood, pathogen, and other infectious agents. Additionally, instruments and supplies that are not heat-resistant or prone to deformation and breakage during processing for reuse should be disposable. Despite this, some dental clinics have reported reuse dental disposables, with 30% of them reusing saliva ejectors (1).

Saliva ejectors are part of the suction systems used to remove aerosols in dental settings, along with other devices such as high-volume evacuators (2). They are commonly used in dental procedures to reduce the spread of biological aerosols, containing infectious agents (3). These instruments are instrumental in reducing the aerosols and splatter generated during dental treatments, thereby enhancing infection control measures (4). Research has shown that when saliva ejectors are used alongside suction devices, they can effectively reduce the dissemination of aerosols and splatter during dental procedures (3, 5). Furthermore, their utility extends to airway management to reduce provider exposure during intubation, showcasing their versatility in healthcare settings (6).

Studies have emphasized the significance of using disposable saliva ejectors along with other techniques such as rubber dam isolation and four-handed dentistry to decrease the risk of transmission during dental procedures (7). The disposable nature of saliva ejectors ensures that each patient receives a new, uncontaminated device, thereby improving infection control practices (8). Additionally, the use of disposable saliva ejectors has been recommended to reduce the spread of biological aerosols in dental settings, thus creating a safer environment for both dental practitioners and patients, particularly in the context of the COVID-19 pandemic (3, 9).

The reuse of saliva ejectors in dental settings has both advantages and disadvantages. The environmental impact of reusable versus single-use medical equipment has been studied, showing cost savings with reusable equipment but increased emissions (10). Disposable saliva ejectors are discarded after a single use. However, there have been reports of the reuse of salivary ejectors in dental clinics. Therefore, it is essential to consider the potential risks associated with reusing salivary ejectors. Improper sterilization or maintenance of reusable ejectors can lead to cross-contamination and the spread of infections in dental settings. Therefore, this study aimed to investigate and compare various disinfection and sterilization methods for contaminated salivary ejectors to investigate the microbiological aspects of salivary ejectors reuse.

2. Materials and Methods

2.1. Selection and culture of experimental bacterial strains

Streptococcus mutans (*S. mutans*) was used to contaminate the saliva ejector (Asa Dental, Massarosa, Italy) to simulate the contamination in a reuse scenario. *Streptococcus mutans* was chosen for the contamination experiment due to its significance in oral health and its role as a contamination marker (11). Bacterial strains were sourced from the Korean Collection for Type Cultures (KCTC) (KCTC 3065). Based on previous study, *Streptococcus mutans* was cultured by inoculating it into 10 ml of Brain Heart Infusion (BHI, Bacto™ Brain Heart Infusion, Maryland, USA) liquid medium. The culture was then incubated at 37°C in an incubator (IB3-05A, Jeio tech, South Korea) for overnight. After incubation, the culture was diluted to an optimal density of 0.600 (Optical Density, OD 600 nm) for optimal

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growth. The bacterial growth was measured using UV/Vis Spectrophotometer (Ubi-490, MicroDigital Co., Ltd., Seongnam, South Korea) (12).

2.2. Experimental group after contamination of saliva ejectors

The saliva ejectors were immersed in a container containing 250 ml of the liquid medium for 5 min. The saliva ejectors were then divided into five groups for further treatment. In Group 1, the contaminated saliva ejectors were washed under running water for 10 seconds using only a brush (without detergent, only brush). In Group 2, the contaminated saliva ejectors were immersed in a chemical disinfectant (Gzyme^{3}, Germiphene Corperation, Brantford, Canada) for 10 min, then were washed for 10 seconds under running water. In Group 3, the contaminated saliva ejectors were first submerged in a chemical disinfectant for 10 min, then were washed for 10 seconds under running water, and finally sterilized using an autoclave (HS-3041VD, Hanshin Medical, South Korea). New saliva ejectors were used as negative controls, while *Streptococcus mutans*-contaminated saliva ejectors were used as positive control. Physical changes in autoclaved saliva ejectors were visually observed, and images were captured using a smart phone camera (iPhone 13 Mini [A2628], Apple Inc., USA). All experiments were conducted in triplicate by the same operator.

2.3. Adenosine triphosphate (ATP) test

To assess surface contamination, an ATP test was conducted to quantify adenosine triphosphate enzyme levels based on the bioluminescence reaction of bacteria metabolism (13). Samples were obtained using an Ultrasnap™ ATP swab (Hygiena LLC, Camarillo, USA) by rubbing the saliva ejector tip and the inside of the tube for 30 seconds. Subsequently, the swabs were placed in a test tube and shaken for 5 seconds. The test tube was inserted into an ATP meter (SystemSURE plus™ ATP Luminometer, Hygiena LLC., Camarillo, USA) to measure the surface ATP of the specimen. Relative light unit (RLU) values were used to compare the experimental groups.

2.4. Microbial culture test

Saliva ejectors from each group were placed in a tube containing 10 ml of phosphate-buffered-saline (PBS) solution (BIONEER Corp., Daejeon, South Korea) and shaken for 10 seconds. PBS containing *Streptococcus mutans* was the added to the BHI liquid medium from the tube in which the saliva ejectors were immersed. The solution containing *Streptococcus mutans* in PBS was mixed with the BHI liquid medium. The mixed solution was serially diluted (10^{-5}) and then inoculated onto BHI agar medium (Difco™ Agar, Maryland, USA) using glass spreader rods on a 60 mm diameter plate (Daihan, South Korea), and also inoculated into BHI liquid medium for further analysis. After incubating at 37℃ aerobically for 24 hours, the bacterial plate images were captured using Quantitative Light-induced Fluorescence-Digital (QLF-D Biluminator 2, Inspektor Research systems BV, Amsterdam, The Netherlands) and a digital single-lens reflex camera (Canon EOS 650D, Japan). Meanwhile, the colony forming units (CFUs) were counted visually. The measurements are presented in the graph as colony counting of bacterial growth.

2.5. Statistical analysis

Each group was tested for normality using the Shapiro-Wilk test. One-way ANOVA was performed for each experimental group to assess the difference in contamination and microbial counts. Post-hoc analysis was performed

using Bonferroni's method. All statistical analyzes were carried out SPSS (IBM SPSS Statistics 26, IBM Corp., Newyork, USA) with the significance set at 0.05. Figures were generated using GraphPad Prism version 8.

3. Results

3.1. Comparison of surface contamination

Three saliva ejector samples from each group (n=15) were analyzed. Surface contamination was assessed using the ATP test to evaluate the differences in contamination and microbial presence when reusing single-use saliva ejectors. Figure 1 presents the comparison of surface contamination. Group 1 was observed to have higher surface contamination compared with two other groups; however, statistically no significant differences were observed among the groups compared to negative control, except the contamination group that served as a positive control.

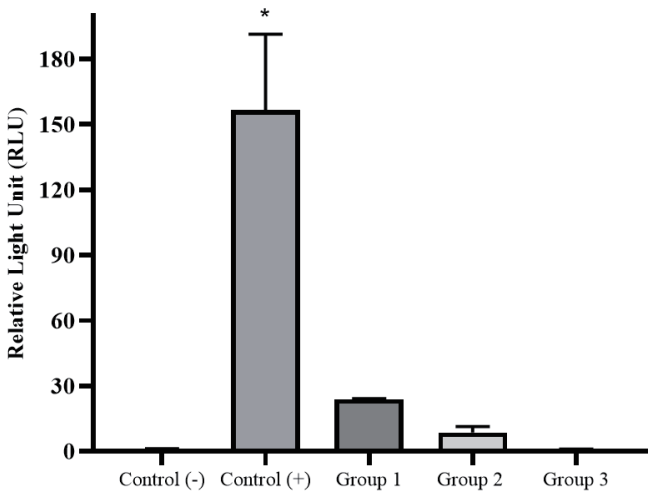


Figure 1. Comparison of surface contamination. New saliva ejectors: negative control; *S. mutans*-contaminated saliva ejectors: positive control; saliva ejectors cleaned with a toothbrush: Group 1; saliva ejectors disinfected with a chemical disinfectant: Group 2; saliva ejectors sterilized with autoclave: Group 3. (RLU, One-way ANOVA compared to negative control, *P<0.05).

3.2. Comparison of bacterial growth

A microbial culture test was conducted after incubation to assess the difference in contamination and microbial growth with reused disposable saliva ejectors. Figure 2 demonstrates significant differences between the new saliva ejector as control and saliva ejector cleaned with a toothbrush only (Group 1), as well as those cleaned with chemical disinfectants (Group 2). In Group 3, which was sterilized using an autoclave, no microbial growth was observed (Figure 2A). In addition, the bacterial relative growth of Group 3 was not significantly difference with negative control. Notably, shrinkage and damage to the salivary ejectors were observed during autoclaving (Figure 3).

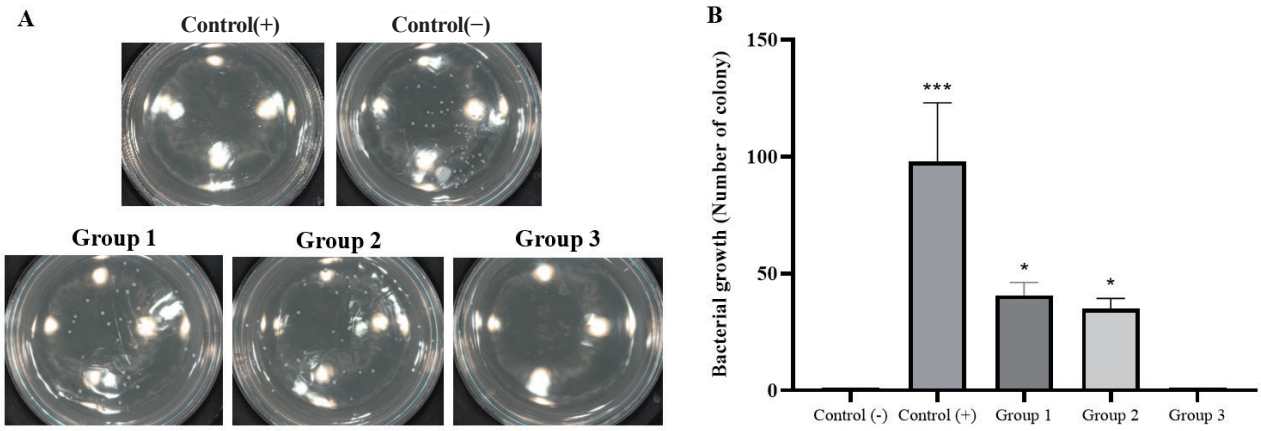


Figure 2. Comparison of bacterial contamination. A. Bacterial growth images by quantitative light-induced fluorescence device. B. *Streptococcus mutans* growth of colony forming unit (CFU) among intervention groups. Data are expressed as bacterial growth (number of colony). PBS: negative control; *S. mutans* contamination: positive control; cleaning using toothbrush: Group 1; chemical disinfection: Group 2; autoclave sterilization: Group 3. One-way ANOVA compared to negative control, **P*<0.05 , **P*<0.01, ****P*<0.001.

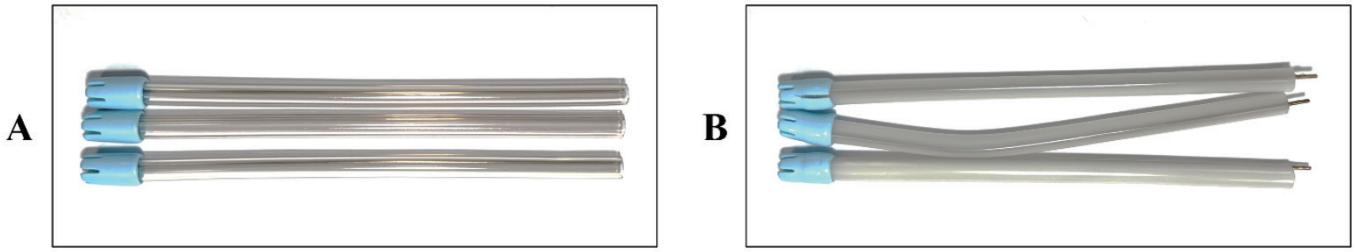


Figure 3. Comparison between new and autoclaved saliva ejectors. Shrinkage and deformation of the saliva ejector by autoclaving were observed. A. New saliva ejector. B. Autoclaved saliva ejector.

4. Discussion

This study aimed to examine and compare various common techniques for sterilizing and disinfecting contaminated saliva ejectors. Previous research has highlighted the reuse of dental instruments in some Korean dental clinics, including 30% of which are salivary ejectors (1). Cost reduction likely being the major factor in this practice. McGain F et al.(10) demonstrated the cost-effectiveness of reusable hospital equipment compared to single-use instruments; however, processing disposable saliva ejectors raises significant hygiene concerns in dental settings.

This study investigated the efficacy of standard disinfection and sterilization methods to assess whether they can achieve a level of disinfection comparable to new-single-use ejectors. The findings of this research are essential for establishing infection control protocols and ensuring patient safety in dental settings, particularly where economic factors may incentivize the reuse of disposable instruments.

The ATP method was employed as a rapid assay to quantify the ATP levels based on bioluminescence. This reaction involves luciferase enzyme, luciferin substrate, and ATP, and the light emitted during the reaction can be quantitatively measured using luminometer (13). This method is commonly utilized in Sweden to complement

visual assessments for monitoring hospital cleanliness (14). The use of ATP measurements has also been linked to a reduction in the spread of multidrug-resistant bacteria, highlighting its important in infection prevention and promoting patient safety (15). In this study, we observed no significant difference among the saliva ejectors groups for cleaning, disinfecting, and sterilizing based on ATP test that was conducted immediately after contamination. These findings indicated the need for further investigation to assess the sustainability of reused saliva ejectors after cleaning and disinfection.

Our findings underscored the critical concerns associated with the use of salivary ejectors. Although cleaning and disinfection procedures initially reduced the microbial load, significant regrowth was observed on saliva ejectors after 24 hours compared to new, unused controls. This suggests that current decontamination methods may not adequately prevent microbial accumulation over extended periods. The porous nature of some disinfectants allows residual moisture and organic matter to persist on their surfaces, creating a conducive environment for microbial regrowth (16). Moreover, the intricate design of saliva ejectors, characterized by narrow lumens and difficult-to-access areas, presents significant challenges for thorough cleaning and disinfection (1). The inability to achieve complete sterilization can promote microbial adherence and biofilm formation, thereby heightening the risk of contamination (16).

Furthermore, our results demonstrate that saliva ejectors cleaned with a disinfectant solution and subsequently sterilized using an autoclave exhibit no microbial growth. This finding is consistent with the recognized effectiveness of autoclaving in eliminating a broad spectrum of microorganisms, including bacteria, viruses, and fungi (17). Due to its capability to achieve sterility, autoclaving remains the gold standard for the sterilization of dental and medical instruments.

However, our study also identified challenges associated with the reprocessing of disposable saliva ejectors. While autoclaving effectively reduces the microbial burden, it occasionally results in shrinkage and damage to the ejectors, potentially compromising their functionality. These observations support existing recommendations for against reusing disposable saliva ejectors (1). Furthermore, previous studies have highlighted the risks of incomplete sterilization and potential cross-contamination associated with reprocessing disposable instruments (3, 7, 8). Sajjanshetty S et al.(18) demonstrated that although autoclaving achieved the greatest reduction in microbial burden among the methods tested, none of the decontamination procedures were completely effective in eliminating all microorganisms from the dental equipment. Collectively, these findings underscore the importance of adhering to infection control protocols that mandate the use of disposable saliva ejectors to ensure patient safety.

Nevertheless, this study had some limitations. First, the use of rapid ATP tests in microbial experiments may lead to fluctuations in ATP levels and an inability to distinguish between live and dead microorganisms, resulting in an inaccurate representation of the actual microbial load (19, 20). Moreover, the sensitivity of ATP tests to environmental conditions such as pH, temperature, and chemical interference can affect the reliability and reproducibility of ATP measurements in microbial experiments (16). These limitations highlight the need for careful interpretation and consideration when using ATP tests in microbial studies. Future studies should incorporate complementary methods to provide a more comprehensive assessment of the microbial burden on salivary ejectors after reprocessing.

Secondly, our study utilized only a single bacterial species. Although *Streptococcus mutans* is important in dental caries development, the oral microbiome is a complex and diverse community. Incorporating a wider range of

microorganisms with varying pathogenicities and environmental tolerances would offer a more comprehensive understanding of the efficacy of various sterilization techniques on the overall microbial ecology of salivary ejectors.

5. Conclusion

In this study, we investigated common methods for disinfecting and sterilizing saliva ejectors contaminated with *Streptococcus mutans*. Our findings revealed that immediately after cleaning and disinfection, no detectable surface contamination was observed on the saliva ejectors. However, over time bacterial proliferation was confirmed. Notably, sterilization using autoclaves yielded the best results in terms of reducing microorganism growth, suggesting its susceptibility for microbiological reuse of saliva ejectors. Nonetheless, the potential for shrinkage and damage resulting from autoclaving, which may affect functionality, should also be considered.

As this study focused exclusively on a single strain of *Streptococcus mutans* strain, future research should aim to include a broader range of contaminants to better mimic the conditions in dental settings. Further studies exploring alternative methods for assessing the reusability of dental instruments are required.

Author contributions

Conceptualization: JY Kang. Data curation: SG Lee, SY Kim, JH Song and GY Kim. Formal analysis: S Prismasari and HY Mun. Funding acquisition: JY Kang. Investigation: HY Mun, SG Lee, SY Kim, JH Song and GY Kim. Methodology: HY Mun and JY Kang. Supervision: JY Kang. Validation: JY Kang. Writing—original draft: S Prismasari, HY Mun and JY Kang. Writing—review & editing: S Prismasari and JY Kang.

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